Biochemistry 441 **2nd Hour Exam** **March 3, 2006**

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2	
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Total	

1. A. Individuals with familial hypercholesterolemia synthesize cholesterol rapidly even though the concentration of cholesterol in their blood is abnormally high. Most people with this condition are found to have genetic defects in their LDL receptors. Why does regulation of cholesterol biosynthesis fail in this situation? (5 points)

Cholesterol travels in the blood mainly in low-density lipoproteins (LDL), which must bind to LDL receptors in order to be taken up by cells in peripheral tissues. The control systems for cholesterol biosynthesis sense the intracellular cholesterol concentration, not the concentration in the blood. For example, expression of the gene for HMG-CoA reductase is triggered when cholesterol dissociates from a protein that binds to the sterol regulatory element in the ER membrane. If a defect in the LDL receptor prevents cholesterol uptake, the intracellular cholesterol concentration will remain low and HMG-CoA reductase, the controlling enzyme for cholesterol synthesis, will be expressed at high levels. Synthesis of the LDL receptor also will remain activated for the same reason, and protolysis of HMG-CoA reductase will remain switched off.

B. You find a strain of mice with hereditary hypercholesterolemia who have normal LDL receptors and normal amounts of HMG-CoA reductase in their tissues. On ion-exchange chromatography, which can resolve proteins with different net charges, HMG-CoA reductase purified from these mice migrates in a single band whereas the enzyme from normal mice separates into two bands. Suggest an explanation for these observations based on what you know about the control of cholesterol biosynthesis. (5 points)

HMG-CoA reductase also is regulated by phosphorylation. Phosphorylation inactivates the enzyme; dephosphorylation activates it. Phosphorylation changes the charge of the protein, so the two bands seen with HMG-CoA reductase from normal mice could be the phosphorylated and unphosphorylated forms. A mutation that prevented phosphorylation (e.g., substitution of a different amino acid for the residue that normally gets phosphorylated, substitution of a residue that participates in binding of the kinase, or a mutation in the kinase) would give an enzyme that migrates as a single band and remains activated.

2. A patient's urine has an unusually high concentration of phenylpyruvate, but his/her phenylalanine hydroxylase activity is within the normal range. You therefore suspect a deficiency in an enzyme that reduces dihydrobiopterin to tetrahydrobiopterin. What's your reasoning? (10 points)

Phenylalanine hydroxylase is a mixed-function oxidase that oxidizes tetrahydrobiopterin along with phenylalanine. (The reaction is Phe + O_2 +THB → Tyr + H_2O + DHB, where THB and DHB are tetra- and dihydrobiopterin, respectively.) If the oxidized product (dihydrobiopterin) cannot be reduced, oxidation of Phe to Tyr will stop. (The intracellular concentrations of tetra- and dihydrobiopterin probably are much lower than the concentrations of Phe and Tyr, since these are co-enzymes, not major metabolic intermediates.)

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3. Draw a metabolic scheme showing how acetyl-CoA produced in the mitochondrial matrix moves to the cytosol for fatty acid synthesis. The scheme should include a mechanism for generating NADPH in the cytosol, and should indicate the roles of oxaloacetate, malate, citrate, pyruvate and ATP in these processes. You don't need to draw the structures of the intermediates; just give the names if you like. (10 points)

Mitochondrion | Inner Membrane | Cytosol

oxaloacetate + acetyl-CoA →
CoA-SH + citrate

citrate + CoA-SH + ATP → ADP + Pi
+ oxaloacetate + acetyl-CoA

NADH
NAD⁺

malate

NADP⁺
NADPH

pyruvate

pyruvate

ADP + P_i
CO_2 + ATP

oxaloacetate

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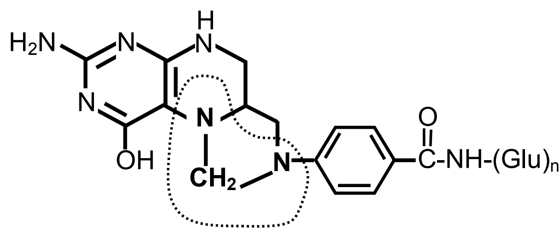
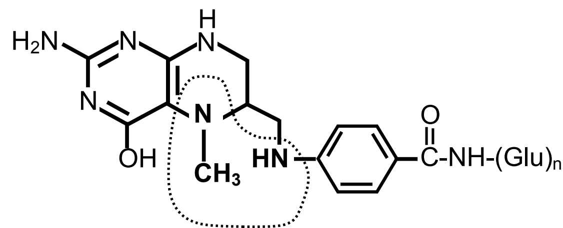
4. Complete the structures of (A) N^5N^{10}methylene-THF and (B) N^5-methyl-THF below. The missing atoms and bonds are all within the dotted lines. (10 points)





A. N^5N^{10}methylene-THF **B.** N^5-methyl-THF

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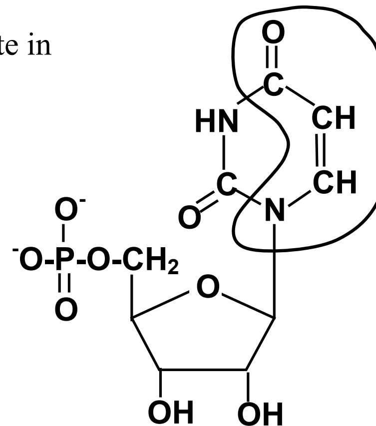
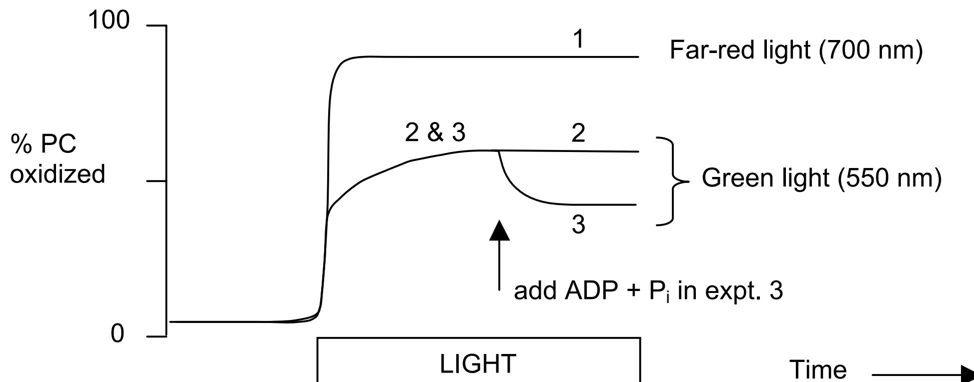
5. Circle the atoms that are derived from aspartate in the structure on the right. (10 points)



6. The graph below shows the extent of oxidation of plastocyanin (the Cu-protein that reduces $P700^{+}$) when chloroplasts were illuminated with either far-red (experiment 1) or green (expts. 2 and 3) light. The box below the curves indicates the period when the light was on. In expt. 3, ADP and Na_2HPO_4 (P_i) were added at the time indicated by a vertical arrow. The light intensities (photons/cm^2/second) were the same in all three experiments.



A. Why is plastocyanin more oxidized in far-red light (expt. 1) than in green (expt.2)? (5 points)

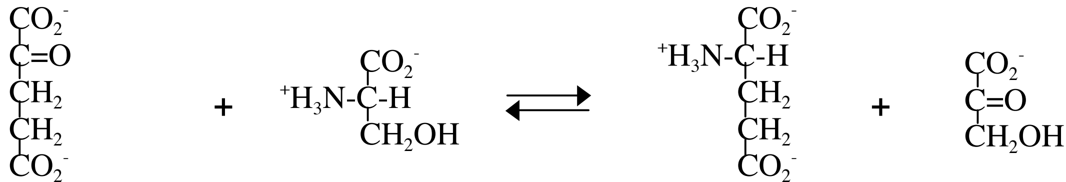
Far-red light drives photosystem I (PS I) but not photosystem II (PS II). Illumination with far-red light therefore leads to oxidation of all the components between PS II and PS I in the Z-scheme, including plastocyanin (PC). Green light drives both photosystems, and so puts PC in a steady state in which the oxidation level reflects the relative rates of oxidation (by PS I) and reduction (by PS II).

B. Why does adding ADP + P_i cause plastocyanin to become less oxidized in expt. 3? (5 points)

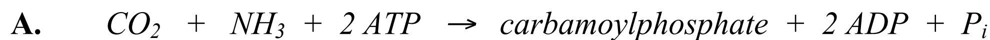
Electrons flowing from PS II to PS I pass through the cytochrome-b_6f complex, where oxidation and reduction of plastoquinone pump protons from the stromal side of the thylakoid membrane into the thylakoid lumen. This creates an electrochemical potential gradient across the thylakoid membrane, with the lumen becoming more acidic and positively charged relative to the stroma. As the electrochemical potential gradient builds up, it creates a thermodynamic back pressure that makes proton pumping progressively more difficult, and this limits the rate of electron flow through the cytochrome-b_6f complex. The rate constant for reduction of PC therefore decreases, shifting the steady-state in the direction of oxidation. (Note the slow phase in the rise kinetics of traces 2 and 3.) When ADP and P_i are added, protons can flow out of the thylakoid lumen through the proton-conducting ATP synthase as ATP is formed, reducing the

electrochemical potential gradient across the membrane. Electron flow through the cytochrome-b_6f complex to PC accordingly speeds up, shifting the steady-state in the direction of reduction. (This was a difficult question. Hats off to those who got it!)

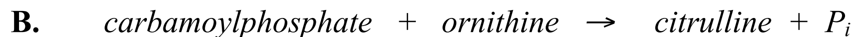
7. Draw the structures of reactants and products of the transamination reaction in which α-ketoglutarate and serine are the starting materials. (10 points)



8. Write the two reactions of the urea cycle that occur in mitochondria. Give either the names or the structures of all the reactants and products in each reaction. (10 points)



A. $CO_2 + NH_3 + 2\ ATP \rightarrow carbamoylphosphate + 2\ ADP + P_i$



B. *carbamoylphosphate + ornithine → citrulline +* P_i

9. Why does a genetic deficiency in adenosine deaminase result in impaired DNA synthesis? (10 points)

Adenosine deaminase catalyzes conversion of adenosine to inosine and deoxyadenosine to deoxyinosine. In its absence, dATP (deoxyadenosine triphosphate) accumulates. dATP inhibits ribonucleotide reductase at the activity regulation site, decreasing the rates of formation of all the deoxyribonucleotides and shutting off the supply of substrates for DNA synthesis.

10. Describe two types of regulation of the bacterial enzyme glutamine synthetase and explain briefly why this enzyme requires a complex regulation mechanism. (10 points)

Glutamine synthetase is controlled by both allosteric effects and covalent modification. The enzyme is inhibited allosterically by the end-products of multiple biosynthetic pathways that branch out from Gln, including carbamoylphosphate, glucosamine-6-phosphate, His, AMP, CTP and Trp. Ala and Gly also inhibit the enzyme. The control by covalent modification involves adenylylation (attachment of a 5'-AMP group as a phosphate ester) of a Tyr residue. Adenylylation, which inactives glutamine synthetase, is stimulated indirectly by Gln (the enzyme's product); de-adenylylation, which activates the enzyme, is stimulated indirectly by Glu (the substrate). Adenylylation and de-adenylylation are mediated by separate enzymes, which are themselves regulated by covalent modification.

A complex control mechanism is necessary because, as stated above, Gln serves as the source of N for biosynthesis of a variety of compounds. Each of the end products alone causes only a partial inhibition of the enzyme, allowing Gln production to continue at a rate sufficient to supply the other pathways. The concentrations of Gln and Glu control the overall availability of Gln for all the pathways. (One can envisage other control mechanisms that might work just as well or possibly even better, such as feedback inhibition of the reactions that branch out from Gln, rather than inhition of glutamine synthetase.)